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Hard Water and MgSO4 on Rabbit Atherosclerosis

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Effect of Hard Water and $MgSO_4$ on Rabbit Atherosclerosis

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Schroeder[1] recently reported the effect that soft drinks and the states and large cities with the hardest water had a lower mortality rate for coronary heart disease than states and large cities with softer water. They also found from their statistical studies that there were negative correlations (protective effect with contents of magnesium, calcium, bicarbonate, sulfate, fluoride, and dissolved solids, the specific conductivity of water, and especially with pH). No significant correlations were found between death rate and contents of silica, iron, sodium and potassium, chloride, the ratio of sulfate to bicarbonate, nitrate, manganese, carbonate, color, turbidity, or the number of treatments given water. There was no correlation with diseases other than cardiovascular disease.

Vitale and his associates[2,3] found that magnesium oxide (MgO) given orally caused a reduction in atherosclerosis in rats on a high-fat diet. They also found that a high-fat diet caused magnesium deficiency in rats not given more magnesium than the usual requirement on a regular diet. They also found the magnesium had no effect on blood cholesterol and that the magnesium-fed rats were larger than the others.

Before the present experiments described here, we did several experiments lasting two months each, using New Zealand rabbits fed on rabbit pellets and 1 percent cholesterol. Our results showed only very minimal atherosclerosis of the rabbit aortas. In these rabbit experiments, which showed very minimal atherosclerosis, we had used hard sulfur water from one of our farm wells. We saw that results different waters have on rabbit atherosclerosis, we did the experiments described below.

Materials and Methods

For the first experiment, we used New Zealand rabbits divided into four main groups of four rabbits each. All four groups were given Purina rabbit pellets, 1 percent crystalline cholesterol, and 5 percent butter. Group 1 was given distilled water. Group 2 was given untreated hard sulfur water from our artesian well. Group 3 was given hard chlorinated water (Jacksonville City Water); see Table 1. This water is obtained from deep artesian wells and aerated to remove the H2S. The different analyses on Table 1 are from the different wells that combine to supply the city. Group 4 was given hard water from the thoracic aorta and rock well. We gave rabbits Sulfa-Nox (solution of sulfaquinoxaline sodium), one teaspoonful per rabbit mixed in the drinking water on the first day of the experiment to prevent illness.

After two months, the rabbits were killed, and the atherosclerosis of the aortas was graded. We used the entire thoracic and abdominal aortas and graded the amount of the atherosclerosis 1+, 2+, 3+, 4+. Representative sections of aortas and coronary arteries were studied microscopically.

Results

The aortas of the rabbits on distilled water had more atherosclerosis than those on hard water. It did not make any difference whether the water was hard sulfur, hard chlorinated, or nonsulfur hard water. The groups on hard water had less atherosclerosis than the group on distilled water. All aortas in all the groups showed some atherosclerosis. There was more in the thoracic aorta than in the abdominal aorta. Sulfa-Nox (solution of sulfaquinoxaline sodium), one teaspoonful per rabbit mixed in the drinking water on the first day of the experiment to prevent illness.

From time to time in the past several years, Dr. Alvan G. Foraker (pathologist, Baptist Memorial Hospital, Jacksonville, FL) and Dr. Lelia H. Wells and Dr. Carl H. Wells, pathologists, Wells Medical Laboratory, Mandall-Taylor Doctor's Building, Jacksonville, have been kind enough to do microscopic examinations on tissue specimens for us. Microscopic studies of the coronary arteries have shown the same degree of atherosclerosis as found in the aorta.

Table 1. City of Jacksonville, FL, Municipal Water Analyses, July 21, 1960

Station	River Oaks	Hendricks	McDuff Ave.	Lakeshore	Main St.	Fairfax	Norwood
Date (1960)	7–6	7–5	7–6	7–6	7–8	7–7	7–8
Time of Sampling	8:45A 10:15A	1:30P 3:00P	11:45P 12:45P	1:30P 2:45P	11:15A 1:40p	2:30P 3:30P	8:45A 9:45A
Pumping rate mgd	5.9	2.2	13.6	3.0	14.2	9.5	5.4
Chlorine feed #/day	44	30	148	35	148	145	72
Chlorine rate #/mg	7.5	13.7	10.9	11.7	21	13.8	13.3
Chlorine rate ppm	.89	1.64	1.3	1.4	1.77	1.74	1.6
Chlorine residual	.75	.87	.6	.35	.75	.45	.70
Hydrogen ion (pH)	7.9	7.77	7.73	7.9	7.7	7.7	7.75
Phenol-phthalein alkalinity	0	0	0	0	0	0	0
Methyl orange alkalinity	115.3	129.3	126	120	147.3	138	141.3
Free carbon dioxide (CO_2)	2.46	4.15	4.07	3.75	4.9	4.6	4
Oxygen (O_2)	5.65	4.03	6.52	5.91	3.62	5.21	4.4
Calcium as $CaCO_3$	199	182	188	188	151	170	143
Magnesium as $CaCO_3$	145	132	134	92	115	105	91
Total hardness	344	314	322	220	266	275	234
Chlorides as Cl	23	27	23	27	27	21	20
Dissolved iron as Fe	0	0	0	0	0	0	0
Sulfate as SO_4	124	369	253	221	209	225	106
Silica as SiO_2	18	19	16	16	21	20	23
No. of samples taken for avg.	3	3	3	3	3	3	3

Analysis made by Plants Efficiency Department, City of Jacksonville, FL (G. Finkba, C. Henley, G. Rones). Hydrogen sulfide exists in all of the above waters prior to treatment in the amount of 1 ½ to 2 ppm. All results in ppm, unless otherwise stated.

Although the main purpose of the experiment was to observe the effect on the rabbit aortas, we did some blood cholesterols on several groups at the end of the experiment. Blood cholesterols of two rabbits of Group 1 (distilled water group) were 2,155 mg percent and 2,050 mg percent. Blood cholesterols of two rabbits in Group IV (nonsulfur water group) were 2,115 mg percent and 1,440 mg percent. The cholesterols were relatively high in both groups.

There has been some criticism of using rabbits for the study of atherosclerosis. Constantinides, Booth, and Carlson[4] have studied rabbits' aortas on a high-fat diet for many months and then allowed to live for two years, they develop lesions similar to those seen in humans. The lesions formed after two or three months are probably similar to fatty deposits seen in young humans.

A second experiment was done to extend the observations on hard water. In the second experiment, we used New Zealand rabbits divided into four groups of five rabbits each. All groups were given Purina rabbit pellets, 1 percent crystalline cholesterol, and 5 percent butter. All rabbits were given Sulfa-Nox (solution of sulfaquinoxaline sodium), one teaspoon per rabbit in the drinking water on the first day of the experiment to prevent illness.

Group I was given distilled water and Group II was given hard sulfur water. To evaluate various elements, Group III was given *drinking* water made by suspending one tablespoon of calcium carbonate in one gallon of distilled water. (This makes a milky suspension in water because calcium carbonate is not very soluble in water.) Group IV was given drinking water made by dissolving seven tablespoons of magnesium sulfate (Epsom salt) in one gallon of distilled water, or 30,000 ppm. This is much more than is usually found in hard water.

Group III on the calcium carbonate water drank more of their water than the Group IV rabbits on magnesium sulfate. This tends to balance some of the difference in concentration. The experiment was continued for three months, then all rabbits were killed and their aortas and hearts were studied. These rabbits were slightly less susceptible to atherosclerosis than the rabbits in the first experiment. This may have been because we obtained these New Zealand rabbits from a different breeder and probably got some hereditary difference. There are variations between individual rabbits, and Gaman and Smith[5] have shown variation between different breeds of rabbits.

Again we found there was more atherosclerosis on the average in the distilled water group (Group I) than in the hard water group (Group II). Group III rabbits (calcium carbonate) showed moderate atherosclerosis (1+ to 2+), while Group IV rabbits (magnesium sulfate) showed no atherosclerosis. The magnesium group did not have diarrhea, and they were larger and fatter than the rabbits in the other groups at the end of the experiment. At autopsy these rabbits had more subcutaneous fat than the rabbits not on magnesium sulfate.

Although the main purpose of the experiment was the study of the amount of atherosclerosis in the arteries, some studies were run at the end of this experiment on blood lipids.

Blood cholesterols of two rabbits in Group I (distilled water) were 885 mg percent and 1,505 mg percent, and both had marked turbidity or "milky" serum. Blood cholesterols of two rabbits in Group IV (magnesium sulfate) were 965 mg percent and 1,300 mg percent, and both had slight turbidity or relatively "clear" serum. Blood cholesterols on two rabbits in Group III (calcium carbonate) were 2,020 mg percent and 2,965 mg percent and both marked turbidity or "milky" serum.

All of these blood serums were very milky except the two magnesium sulfate blood specimens, which were relatively clear. The two distilled water serums (milky) and two magnesium sulfate serums (clear) had similar blood cholesterols, but there was marked difference in the appearance of the serums. To find out if this difference was due to a difference in other fat components, further studies were run on these serums. (See Table 2.)

Table 2. Comparison of Two Typical Blood Serums

	A "Milky," Distilled Water Serum (mg %)	A Relatively "Clear" Magnesium Sulfate Serum (mg %)
Cholesterol	1,505	1,300
Phospholipids	386	433
Triglycerides	120	508
Total lipids	2,011	2,241

Comment

These experiments showed some protective effect of hard water on rabbit atherosclerosis as compared to distilled water. Large amounts of calcium carbonate dissolved and suspended in distilled water gave no protective effect against rabbit atherosclerosis, while large amounts of magnesium sulfate dissolved in distilled water gave complete protection against rabbit atherosclerosis.

The relatively "clear" serum of rabbits drinking distilled water with magnesium sulfate added had essentially the same amount of fat (cholesterol, triglyceride, and phospholipids) as the turbid or milky serum of rabbits drinking distilled water. Vitale[2,3] found similar results in the rat by feeding MgO orally as we did in rabbits feeding $MgSO_4$ orally. They did not mention the appearance of the serum or lipemia, but they found in the rat as we did in the rabbit that there was no effect on blood cholesterol, although there was a reduction in atherosclerosis and the magnesium-fed animals were larger.

When the blood serums from the rabbits in this experiment were allowed to stand for one day, the fat particles settled out in the milky serum, but the fat in the clear serums of the magnesium sulfate rabbits did not settle out. When a slight to moderate amount of magnesium sulfate was added to the milky serums in the test tube there was some clearing of the serum, but when a very large excess of magnesium sulfate was added, there was clouding of the serum. Repeated large closes of magnesium sulfate in humans can have toxic effects.[6]

There are several important questions raised by the experiments reviewed and reported. What is the protective substance or substances in hard water? Is this a nonspecific effect of ions on the stability of colloidal fat? The ability of dispersed particles to maintain the colloidal state is greatly affected by the concentration of ions.[7] Colloid, especially hydrophobic colloids such as colloidal fats, can adsorb ions on their surfaces, which increase their stability at certain ion concentrations. The charged colloids repel each other. Or is this a specific effect of magnesium on fat metabolism? Further studies will have to be done using $CaCO_3$ and other ionizing compounds to answer these questions.

Parsons[8] recently reported a study in patients with coronary artery disease given a restricted-fat diet, with 2 ml of magnesium sulfate and 5,000 units of heparin intramuscularly weekly for up to one year. They found a sharp fall in total *lipids*, *B*-lipoproteins and protein-bound cholesterol. They state that heparin causes a marked clearing of lipemia, and administration of magnesium sulfate reduces coagulability and lowers *B*-lipoproteins while increasing fibrinolytic activity. They suggest that parenteral magnesium sulfate and minimal doses of heparin might be used to treat coronary artery disease. Further study should be done on the effect of these substances separately and combined on blood lipids and atherosclerosis. Electrolytes other than magnesium sulfate should also be studied.

Summary

Several experiments with New Zealand rabbits showed that rabbits on a high-fat, high-cholesterol diet had less atherosclerosis of aortas and coronary arteries if they drank hard water than if they drank distilled water. A group of rabbits on a high-fat, high-cholesterol diet drinking distilled water with calcium carbonate dissolved and suspended in the water had moderate atherosclerosis, while rabbits on a high-fat, high-cholesterol diet drinking distilled water with magnesium sulfate dissolved in the water had no atherosclerosis.

The blood serums from the distilled water rabbits and the calcium carbonate rabbits were very turbid or milky, while the serums from the magnesium sulfate rabbits were relatively clear, although all groups had essentially the same amount of fat in their serums.

We are grateful to Dr. Leila H. Wells and Dr. Carl H. Wells, pathologists, Wells Medical Laboratory, and Dr. Alvan G. Foraker and his associates in the Pathology Department, Baptist Memorial Hospital, Jacksonville, FL, for microscopic studies. We are grateful to Mrs. Sophie Adams, MT, Miss Nancy Franklin, MT, Miss Mary Weakley, MT, Mr. Don Osborne, BS, and Mr. Jack Mahoney, MT, for laboratory studies.

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